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Studies on Development of Resistance in Different Strains of *Trogoderma granarium* (Everts) to Phosphine Fumigation in Southern Punjab, Pakistan

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Abstract: The study was conducted to determine the response of different strains of *Trogoderma granarium* (Everts) to phosphine concentrations. Thepest was exposed to 200, 400, 600 and 800 ppm concentrations of phosphine gas in glass jars under control conditions following CRD, replicated four times along with a control treatment. The observations regarding percent mortality of grubs of *T. granarium* were recorded after 1, 3, 5, 7 and 15 days of application of phosphine gas. The results revealed that maximum mortality of the pest was recorded to be 81.67 % in jars where 800 ppm concentration of the gas was applied 47.55, 61.42 and 70.33% mortality was observed by 200, 400 and 600ppm concentration. Shah Sadardin strain showed maximum mortaility (68.35%) followed by Tounsa Sharif strain (45.95) and D. G. Khan strain (43.60%).

Key words: phosphine, *Trogoderma granarium*, concentrations, exposure intervals.

Introduction

Grain storage is one of the most important tasks confronting the grain handling agencies and the stored grain entomologists of the world today, because of an admitted fact that post harvest losses of grains in Pakistan are 10% – 15% (Jilani, 1981). Main reasons of these losses are: lack of sanitary conditions in and around the storage system, leaky godowns, improper application of fumigants and non-availability of trained manpower in food handling agencies. There is no denying the fact that the biological agents are responsible for major part of the post harvest losses. Insect pests especially coleopterans are very important biological loss causing agents. The stored grain losses by these insect pests have been calculated to be 3.6 to 25.5 % (Irshad and Baloch, 1985). Ahmad (1984) reported that 6.75 million tones of wheat that remained in stores for a period of 6 to 12 months suffered for loss of 0.169 tones due to these insect pests. Among the coleopterous insect pests in storage systems, *Trogoderma granarium* (Everts) is considered to be the world's worst pest of stored grain (Christensen and Kaufmann, 1969). In Pakistan, it is a very destructive pest of wheat and other stored grains, particularly of the North-Western Dry Regions.

Phosphine fumigation is the principal and efficient method to control stored grain insect pests but lack of education and training in grain storage management, resulting in improper exposure periods, gas leakage and sub-lethal concentration make this tool in-effective. The substandard techniques of phosphine fumigation have led to the development of phosphine resistance in major insect pests of stored grains (Mills, 1983; Taylor, 1989; Mills *et at*, 1990). Borah and Chahal (1979) reported that phosphine failed to control khapra beetle, *Trogoderma granarium* in warehouses in India. Tyler *et al.* (1983) documentedthe development of resistance in stored grain insects pests againstuseful insecticide, phosphine, in warehouses in Bangladesh. Appreciably high resistance was recorded in *Trogoderma granarium* strains collected from Punjab and Sindh (Alam *et al.*, 1999).

The present project has been carried out to determine mortality in different strains of *T. granarium* collected from D. G. khan District at different concentrations (200 ppm, 400 ppm, 600 ppm, 800 ppm and 0 ppm as control) of phosphine at different exposure periods. The results of present study provide useful information for the effective management of T. *granarium*.

Materials and Methods

The research project was conducted in the Grain Research, Training and Storage

Management Cell, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan. Large number of live adults and grubs of *T. granarium* were collected from various in-

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fested godowns in Dera Ghazi Khan, district of punjab. Three strains viz., D. G. Khan city, Shahsadardin and Tehsil Tounsa sharif were used for experimentation. These strains were kept in wide mouth glass jars covered with muslin cloth. Each strain was labelled and reared separately in the laboratory for two months. The adults of *T. granarium* of each strain were sieved and placed in breeding containers (wide mouth glass jars) in the medium ofuninfested wheat grains. The jars were placed in Gallenhamp incubator at 35 ± 1℃ and 65% ± 5% relative humidity and were kept there for three days. The insects were removed from the above jars and transferred to new containers. The media in which the adults of the test insect were retained initially for three days contained sufficient number of eggs. The larvae first appeared after four days with the majority appearing after six days. Progenies removed from these stocks were supposed to be of same age. It was further kept for another period of five days. The larvae were allowed to flourish under the optimal development conditions, i. e. 35 ± 1℃ and 65% ± 5% relative humidity.

For phosphine gas generation, a funnel tied with thread was hanged over a cylinder filled with 5% sulphuric acid solution. A tablet of aluminium phosphide (Agtoxin) wrapped in muslin cloth was dropped in the solution in such a away that it went directly under the funnel in the solution. This funnel was tied with the thread below the burrete. The air in the burrete was sucked out with the help of a syringe through rubber septum, until the solution rose in to the burrete up to the mark. Phosphine gas liberated and accumulated in the burrete over acidified water-When it was filled with gas, 5 mL of gas was sucked out with the help of an air tight syringe and was injected into the sealed glass jar of known volume (1125 cc); 50 mL of gas sample from the jar-was taken and injected in to the Harris conductivity meter (Harris, 1986) for measuring gas concentration.

100 grams of wheat grains were taken in each glass jar and amount of phosphine for each glass jar was calculated by using following formula:

Phosphine required = Concentration × Volume of jar × 836.81

A 500μL (micro-litre) syringe was used to inject phosphine concentration into the jars. For each exposure period (1, 3, 7 and 15 days) four concentrations of phosphine were injected into the jars. A complete test (i. e. for single concentration) had four repeats of 25 grubs for each exposure period with one control. Data were collected after each exposure period and corrected mortality calculated by using Abbot's formula as recommended Busvine (1980). Finally the data were analyzed statistically.

Results and Discussion

The results revealed that maximum mortality of the pest was recorded to be 81.67 % in jars where 800 ppm concentration of the gas was applied 47.55%, 61.42% and 70.33% mortality was observed by 200, 400 and 600ppm concentration. Shah Sadardin strain showed maximum mortaility (68.35%) followed by Tounsa Sharif strain (45.95) and D. G. Khan strain (43.60%). It was observed that mortality increased as the concentration of the gas was increased with the increase at all exposure periods as depicted by Table1

Table 1. Comparison of mean values regarding percent mortality of different strains of *Trogoderma granarium* (Everts) against different concentrations of phosphine gas at various exposure periods.

CONCENTRA TIONS (ppm)	POST TREATMENT MORTALITY (%)				AVERAGE
	1 DAY	3 DAYS	7 DAYS	15 DAYS	
0	0.00 m	0.00 m	3.33 1m	5.331	2.17e
200	18.67 k	39.33 h	60.67 f	71.67 e	47.58d
400	22.67 j	60.33 f	75.67 d	87.00 c	61.42 c
600	29.00 i	73.33 de	84.00 c	95.00 b	70.33 b
800	42.67 g	86.67 c	97.33 ab	100.0 a	81.67 a
Average	22.60 d	51.93 c	64.20 b	71.80 a	

Means sharing similar letters are not significantly different by DMR Test at $P = 0.05$

Table 4. b. Comparison of mean values regarding percent mortality of different strains of *Trogoderma granarium* (Everts) in phosphine gas treatments at various exposure periods.

Strains	Post treatment mortality (%)				AVERAGE
	1 DAY	3 DAYS	7 DAYS	15 DAYS	
D. G. Khan	5.801	42.40 h	59.00 e	67.20 d	43.60 c
Tounsa Sharif	7.401	46.40 g	60.20 e	69.80 c	45.95 b
Shah Sadardin	54.60 f	67.00 d	73.40 b	78.40 a	68.35 a
Average	22.60 d	51.93 c	64.20 b	71.80 a	

Means sharing similar letters are not significantly different by DMR Test at $P = 0.05$.

Table 3. LC_{50} values(ppm) of phosphine for different strains of *T. granarium* (Everts) for various exposure periods.

Strains	1 day	3 days	7 days	15 days
D. G. Khan	13058.06	422.567	238.025	185.759
Taunsa Sharif	1491.33	361.828	159.324	91.551
Shah Sadardin	195.721	70.283	65.601	52.747

The results revealed that maximum mortality(100 percent) of the pest was observed at 15 days exposure period under 800 ppm concentration. The results indicated that maximum mortality of the pest was observed at the maximum concentration of the gas with maximum exposure period. All the concentrations and exposure periods differed significantly in response to pest mortality.

Compared with those of Winks et al. (1980) who reported that period of exposure over 10 days proved lethal for stored grain pests. Similarly Borah and Chahal (1979) reported that *T. granarium* had become resistant to phosphine in certain areas. The present findings are also in conformity with those of El – lakwah et al(1989) who reported that the effectiveness of phosphine was reduced for short exposures and increased for longer exposure. A general statement was given by Shroff and Dhuri(1991) that phosphine was an ideal fumigant for the control of storage insect pests. The present findings are in partial agreement with those of Aheer et al. (1993) who tested various fumigants under control conditions @ 30 tablets per 28.3 m^3 and reported 100 percent mortality. The present findings cannot be compared with those of Winks et al. (1980), Ranjendaran(1982), Bell et al. (1984), Desmarchelier and Wohlgemuth (1984), Reichmuth(1985), Bell(1985), Price and Mills (1988), Ahmad (1989), Ali et al. (1989), Udeaan (1990), Ahmad et al. (1993), Molinari et al. (1993), Muller (1994), Sharma and Karla (1995), Bell and Wilson 1995), Gibe et al. (1997), Sharma and Karla(1998), Sarfraz et al. (2000), Rajendran et al. (2001), Mordkovich (2002), Gooch (2002), Roels et al. (2002), Dalish et al. (2003) and Qin et al. (2003) because of differences in their materials and methods.

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